J Bras Patol Med Lab, v. 52, n. 4, p. 217-222, August 2016

10.5935/1676-2444.20160036

Application of PCR-SSP method for *HLA-B*27* identification as an auxiliary tool for diagnosis of ankylosing spondylitis

Aplicação da metodologia de PCR-SSP na identificação de HLA-B*27 *como auxílio ao diagnóstico de espondilite anquilosante*

Gisele Cristina A. Oliveira; Eliane P. Ambrosio-Albuquerque; Jeane Eliete L. Visentainer

Universidade Estadual de Maringá (UEM), Paraná, Brazil.

ABSTRACT

Introduction: Human leukocyte antigens (HLA) are molecules that present antigen to the immune system; their presence or absence have been described as an influential factor in some diseases. *HLA-B*27* is an HLA polymorphism that has been associated with increased susceptibility to ankylosing spondylitis (AS) and other spondyloarthropaty. The detection of *HLA-B*27* has been used as diagnostic and prognostic tool in these cases, as well as in the differential diagnosis of other diseases. **Objective:** Standardize the single specific primer-polymerase chain reaction (PCR-SSP) methodology for use in the Immunogenetics Laboratory of the Universidade Estadual de Maringá (UEM), considering its specificity and cost-effectiveness. **Material and methods:** A total of 30 individuals without AS positive for *HLA-B*27* allele and 10 AS negative individuals, were previously tested by PCR-sequence specific oligonucleotide (PCR-SSO) and, in this study, by PCR-SSP. **Results:** One hundred percent of the patients tested confirmed their results, even with different subtypes. **Conclusion:** Considering the high reproducibility and the broad spectrum of subtypes covering, it was concluded that the PCR-SSP identifying method for *HLA-B*27* can be used as a routine diagnostic tool for spondyloarthropathies.

Key words: *HLA-B*27* antigen; ankylosing spondylitis; molecular biology.

INTRODUCTION

The major histocompatibility complex is a set of mapped genes on the short arm of chromosome 6, which encodes molecules presenting antigens to the immune system, called human leukocyte antigen (HLA)[1]. HLA molecules are divided into classes: in class I region are loci A, B and C[2], that generates products with high degree of allelic variation at each locus, resulting in a large variability in individuals[3]. The presence or absence of some of these antigens has been related to some diseases[1]. By August 2015, 3,977 *HLA-B* alleles were identified in different populations[4].

The *HLA-B*27* is a polymorphism of the *HLA-B*, described in 1969[5]. The presence of this allele was associated with ankylosing spondylitis (AS) and other spondyloarthropathies[6-8], a group of chronic inflammatory diseases with clinical and radiological manifestations in common[9]. About 184 subtypes of this *HLA*[10] are known, and *HLA-B*27:05* is the most widely distributed and probably is the allele from which others have developed[11-14].

The *HLA-B*27* connection to the AS is well established; 90%-95% of patients with the disease have this allele[15-18], suggesting a tendency to family association[19-21]. The prevalence of AS accompanies the frequency of *HLA-B*27* allele in the population[22], however studies indicate that only 2% of the individuals *HLA-B*27* positive develop the disease[23], indicating the involvement of other genetic and/or environmental factors in the onset of it[24, 25]. The exact trigger mechanism has not been identified[26].

Moreover, some studies indicate that certain subtypes have a greater association with AS than others, suggesting clinical

First submission on 19/05/16; last submission on 19/05/16; accepted for publication on 28/06/16; published on 20/08/16

specificity[27-32]. The *HLA-B*27:05* and the *HLA-B*27:02* are the most common subtypes, 90% and 5%-10%, respectively, in Caucasians[33]; the *HLA-B*27:04* is the predominant subtype among Chinese and Japanese[34] and the *HLA-B*27:04*, the *HLA-B*27:06* and the *HLA-B*27:07* are found only in Asia[35, 36]. This allele is rare in black African populations, as well as the frequency of AS in this ethnic group. In Brazil, the disease is found in mulattos, since they have the Caucasian genetic influence[37].

The *HLA-B*27* has been used as a diagnostic marker for AS and other spondyloarthropathies[38, 39]. The presence of the allele is not linked to the development of AS, but its presence may be useful in the diagnosis and also to auxiliate in the differential diagnosis of other diseases[2]. Furthermore, it can be used as indicator of patient prognosis; individuals with AS and *HLA-B*27* tend to have more prolonged and severe symptoms[40].

The identification of *HLA-B*27* and its subtypes is commonly performed by polymerase chain reaction-sequence specific oligonucleotide (PCR-SSO) and polymerase chain reaction-sequence specific primer (PCR-SSP) methodologies. This is a method considered fast, efficient and relatively low cost[41]. The advantage is the differentiation of several alleles[42], this method allows to detect a single different base in the the deoxyribonucleic acid (DNA) sequence between two alleles, though it can not detect a new undefined allele, unless the change happen in the location detected by the primer[43]. The PCR-SSO, when compared to the PCR-SSP, may present more ambiguities because the probes used are able to bind to the DNA of the sample in a single region per test; the PCR-SSP can bind to the DNA of the sample in two regions per test[44].

Due to the benefits of PCR-SSP methodology and clinical applicability of *HLA-B*27*, the standardization of this method for use in routine and research in the Immunogenetics Laboratory of the Universidade Estadual de Maringá (UEM) is justified.

MATERIAL AND METHODS

Case series

We included 30 patients without AS previously tested by PCR-SSO (One-Lambda, low-medium resolution) and proven positive for *HLA-B*27* allele from the National Register of Bone Marrow Donor (Registro Nacional de Doadores de Medula Óssea [REDOME]) of the city of Maringá and surrounding region. The control-group consisted of 10 individuals from the same database, but negative for *HLA-B*27* allele, evidenced by the same methodology.

The selection of individuals in the database considered the heterogeneity of the *HLA-B*27* alleles to identify the greatest number of subtypes. These have been identified, including cross-reactions, by the National Marrow Donor Program (NMDP) code (http://www.marrow-donor.org/cgi-bin/DNA/dnatyp.pl). **Table 1** describes the *HLA-B*27* subtypes tested and confirmed in this study.

TABLE 1 – Positive results for *HLA-B*27* by PCR-SSO and PCR-SSP methods

Patients	NMDP code of subtypes identified by PCR-SSO and PCR-SSP	Possible alleles that the subtypes represent
H1	B*27:AETG	*27:03/27:05/27:13/27:14/27:17*
H2	B*27:ADDM	*27:03/27:05/27:13/27:17/27:19*
H3	B*27:09	-
H4	B*27:BWHH	*27:03/27:05/27:07/27:11/27:13/27:17/27:32*
H5	B*27:TGG	*27:02/27:30*
H6	B*27:02	-
H7	B*27:BXPE	*27:03/27:05/27:13/27:17/27:32*
H8	B*27:CXME	*27:03/27:05/27:13/27:17/27:38*
H9	B*27:CWNS	*27:03/27:05/27:13/27:17/27:32/27:37*
H10	B*27:08	-
H11	B*27:BRRD	*27:03/27:05/27:13/27:17/27:19/27:28*
H12	B*27:AS	*27:07/27:11*
H13	B*27:CFBR	*27:03/27:04/27:05/27:10/27:13/27:14/27:15/27:17*
H14	B*27:CNCS	*27:07/27:11/27:34*
H15	B*27:XME	*40:01/40:10/40:22N/40:43*
H16	B*27:ADDM	*27:03/27:05/27:13/27:17/27:19*
H17	B*27:BWHH	**
H18	B*27:BXPE	**
H19	B*27:AETG	**
H20	B*27:CXME	**
H21	B*27:TGG	**
H22	B*27:BRRD	**
H23	B*27:CWNS	**
H24	B*27:CXME	**
H25	B*27:BXPE	**
H26	B*27:ADDM	**
H27	B*27:BWHH	**
H28	B*27:BRRD	**
H29	B*27:CWXB	*27:03/27:05/27:13/27:17/27:19/27:38*
H30	B*27:PEN	*27:03/27:05/27:13/27:17*

*HLA: human leukocyte antigens; PCR-SSP: polymerase chain reaction-single specific primer; PCR-SSO: polymerase chain reaction-sequence specific oligonucleotide; NMPD: National Marrow Donor Program; **: possible alleles mentioned above.*

DNA extraction

The DNA extraction was performed with the BIOPUR extraction kit (Kit de Extração Mini Spin Plus 250, Biometrix), using from 200 µl of whole blood collected with ethylenediaminetetraacetic acid (EDTA). After extraction, the DNA was quantified in Nanodrop (NanoDrop 2000 Spectrophotometer, Thermo Scientific-Uniscience).

PCR-SSP

The primers specific for *HLA-B*27* were selected from sequences of the International ImMunoGeneTics Database (IMGT)/HLA (http://www.ebi.ac.uk/ipd/imgt/hla/probe.html) and described by Kulkarni *et al.*[45]; the sequences are shown in **Table 2**. The Amplicon flanked by these primers was designed to identify the *HLA-B*27* subtypes from *27:01* to *27:73* and expand the identification, an additional primer was included[46]. The *HgH* gene was used as an internal control of the reaction.

TABLE 2 – Specific primers for *HLA-B*27*

Sense	Sequence
Sense	1-5' -GCTACGTGGACGACACGCT-3'
Antisense	1-5' -CTCGGTCAGTCTGTGCCTT-3'
Antisense	2-5' -TCTCGGTAAGTCTGT GCCTT-3'
HgH sense	-5'-TGCCTTCCCCAACCATTCCCCTTA-3'
HgH antisense	5'-CCACTCACGGATTTC TGTTGTGTTTG-3'

HLA: human leukocyte antigens.

The conditions of the reaction used were described by Parasannanavar *et al.* (2013)[47] with modifications. The tests were initiated at the following conditions: 1 µl buffer, 0.5 µl magnesium chloride ($MgCl_2$), 0.5 µl deoxyribonucleotide phosphates (dNTP), 2.5 µl primers and 0,5 µl Taq, this volume was used for five samples. The amplifications were performed in a thermocycler (Veriti 96 Well Thermal Cycler, Applied Biosystems) at settings: initial denaturation at 94ºC for 5 minutes, 30 cycles at 94ºC for 1 minute, at 65ºC for 2 minutes, at 72ºC for 1 minute and final extension at 72ºC for 10 minutes. The amplification products were subjected to electrophoresis (Electrophoresis Power Supply LPS – 300 V, Loccus Biotecnologia) in 2% agarose gel with 0.5 µg/ml Syber safe and bromophenol blue in 0.5× buffer of tris-borate-EDTA (TBE) at 80 V for 30 minutes. The identification of bands was performed using a transilluminator (Transiluminador UVB LTB 20 × 20 STV, Loccus Biotecnologia); the band corresponding to the *HgH* gene has 434 pb and the positive band for *HLA B*27*, 149 pb.

RESULTS

To standardize the PCR-SSP, tests were performed with several amounts of each compound to improve the visibility of the bands in the gel testes. In the first mix tested, the reaction was not satisfactory, the control bands were visible, but the bands of the *HLA-B*27* were poorly visible. Modifications and sequential test were conducted by modifying a test variable to reach the best reaction condition, which is composed of: 12.5 µl buffer, 4 µl $MgCl_2$, 2.5 µl dNTP, 1.5 µl primers, 1 µl primers of *HgH* gene and 1.25 µl Taq. Moreover, cycling conditions were also changed to improve the visibility of the bands in the agarose gel, reaching the optimum file of: initial denaturation at 94ºC for 5 minutes, 30 cycles at 94ºC for 40 seconds, 64ºC for 1 minute, 72ºC for 1 minute and final extension at 72ºC for 10 minutes. The **Figure** shows the pattern of bands obtained using the final mix, standardized.

From the standardization, the *HLA-B*27* alleles were tested and all subtypes were amplified by PCR-SSP. **Table 3** shows the results. One hundred percent of sensitivity and specificity were obtained, there was no false positive or negative result.

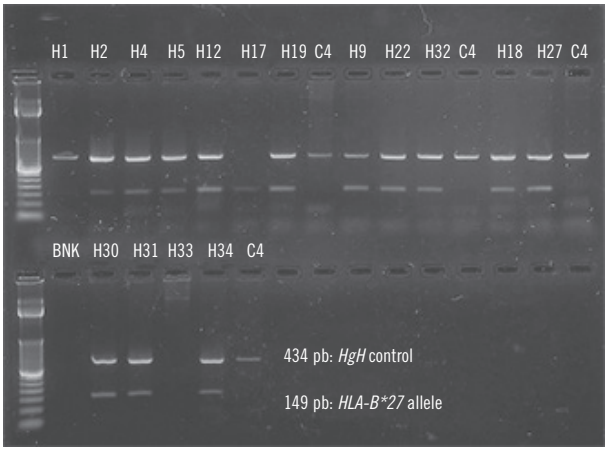

FIGURE – *Band pattern of positive and negative samples for* HLA-B*27 *by PCR-SSP in agarose gel with standardized mix*

PCR-SSP: polymerase chain reaction-single specific primer; HLA: human leukocyte antigens; H: HLA-B*27 *positive; C:* HLA-B*27 *negative; BNK: blank.*
Step ladder: 50 pb.

TABLE 3 – Positive and negative results for PCR-SSO and PCR-SSP

		*HLA-B*27* (PCR-SSO)	
		Present	Absent
PCR-SSP testing	Positive	30	0
	Negative	0	10

PCR-SSO: polymerase chain reaction-sequence specific oligonucleotide; PCR-SSP: polymerase chain reaction-single specific primer; HLA: human leukocyte antigens.

DISCUSSION

Due to the association of the presence of the *HLA-B*27* allele with spondyloarthropathies, the molecular detection requires this allele, which is of utmost importance for both the study and the diagnosis of these diseases. Furthermore, this detection in asymptomatic patients and the research on the risk of patients relatives developing the disease is also essential, that's because epidemiological studies have shown that 10%-20% of first-degree relatives of patients with AS have higher risk of developing the disease[48].

According to Parasannanavar *et al.* (2013)[47], the methods used for identification of *HLA-B*27* (microlymphocytotoxicity and flow cytometry) are difficult because of the lack of a specific antiserum and the possibility of false results due to the change on epitopes. Molecular techniques are more sensitive and specific. The authors tested 90 samples from healthy controls by PCR-SSP and PCR-SSO and found no discrepancy in results between them, data compatible with this study, in which the efficiency of PCR-SSP and the applicability of the primers used was proven, since all results were consistent with those obtained by PCR-SSO, reaching 100% of specificity and sensitivity.

The difficulty encountered was the standardization of the DNA concentration to be used, Parasannanavar *et al.* (2013)[47] suggest the use of 80-100 ng, and this value is not always obtained. Alternative for DNA extraction methods may be used, such as salting out method[49], which expects a higher performance, but requires more time and the use of larger amounts of blood or buffy-coat.

Other authors, such as Frankenberger *et al.* (1997)[50], also found that identification of *HLA-B*27* by PCR is reliable and reproducible, and therefore recommended for the routine, since it overcomes the weaknesses of serology, such as cross-reactivity and ambiguous results.

It was concluded that the PCR-SSP identification method can be used as a routine diagnostic aid for spondyloarthropathies. It is a relatively simple, quick, low costly, high sensitivity and specificity technique.

RESUMO

Introdução: *Os antígenos leucocitários humanos (HLA) são moléculas que apresentam antígenos ao sistema imune; a presença ou a ausência deles é descrita como fator influente em algumas doenças. O* HLA-B*27 *é um polimorfismo do HLA que tem sido associado à maior predisposição à espondilite anquilosante (EA) e a outras espondiloartropatias. A detecção do* HLA-B*27 *é utilizada como ferramenta diagnóstica e prognóstica nesses casos, assim como no diagnóstico diferencial de outras doenças.* ***Objetivo:*** *Padronizar a metodologia de reação em cadeia da polimerase-iniciador específico (PCR-SSP) para utilização no Laboratório de Imunogenética da Universidade Estadual de Maringá (UEM), considerando sua especificidade e seu custo-benefício.* ***Material e métodos:*** *Foram utilizados 30 indivíduos comprovadamente sem EA positivos para o alelo* HLA-B*27 *e 10 indivíduos negativos, testados previamente por PCR-oligonucleotídeo específico (PCR-SSO) e, neste estudo, por PCR-SSP.* ***Resultados:*** *Cem por cento dos pacientes testados tiveram seus resultados confirmados, mesmo com diferentes subtipos.* ***Conclusão:*** *Tendo em vista a alta reprodutibilidade e o amplo espectro de subtipos que abrange, concluiu-se que a técnica de identificação de PCR-SSP para o* HLA-B*27 *pode ser utilizada como auxílio diagnóstico de rotina para espondiloartropatias.*

Unitermos: *antígeno* HLA-B*27*; espondilite anquilosante; biologia molecular.*

REFERENCES

1. Martinez-Borra J, Gonzalez S, Lopez-Larrea C. Genetic factors predisposing to spondylarthropathies. Arthritis Rheum. 2000; 43(3): 485-92.

2. Rudwaleit M. New approaches to diagnosis and classification of axial and peripheral spondyloarthritis. Curr Opin Rheumatol. 2010; 22(4): 375-80.

3. Castro-Santos P, Gutierrez MA, Diaz-Pena R. Genetics of ankylosing spondylitis. Rev Med Chil. 2014; 142(9): 1165-73.

4. International Immunogenetics databases [Internet]. Available at: http://www.ebi.ac.uk/ipd/imgt/hla/stats.html.

5. Thorsby E. HL-A antigens and genes. I. A study of unrelated Norwegians. Vox Sang. 1969; 17(2): 81-92.

6. Brewerton DA, Hart FD, Nicholls A, Caffrey M, James DC, Sturrock RD. Ankylosing spondylitis and HL-A 27. Lancet. 1973; 1(7809): 904-7.

7. Brewerton DA. Discovery: HLA and disease. Curr Opin Rheumatol. 2003; 15(4): 369-73.

8. Schlosstein L, Terasaki PI, Bluestone R, Pearson CM. High association of an HL-A antigen, W27, with ankylosing spondylitis. N Engl J Med. 1973; 288(14): 704-6.

9. Zeidler H, Mau W, Khan MA. Undifferentiated spondyloarthropathies. Rheum Dis Clin North Am. 1992; 18(1): 187-202.

10. Allele Search Tool. IMGT/HLA-IPD-EMBL-EBI. [Internet]. Available at: http://www.ebi.ac.uk/ipd/imgt/hla/allele.html.

11. Khan MA, Ball EJ. Genetic aspects of ankylosing spondylitis. Best Pract Res Clin Rheumatol. 2002; 16(4): 675-90.

12. Khan MA. HLA in spondyloarthropathies. In: Mehra NK, editor. The HLA complex in biology and medicine. A resource book. New Delhi: India Jaypee Brother Med Publ Ltd. 2010; p. 259-75.

13. Ankylosing spondylitis. Ankylosing spondylitis. [Internet]. Available at: http://www.hlab27.com/.

14. Van Gaalen FA. Does HLA-B*2706 protect against ankylosing spondylitis? A meta-analysis. Int J Rheum Dis. 2012; 15(1): 8-12.

15. Oostveen J, Prevo R, den Boer J, van de Laar M. Early detection of sacroiliitis on magnetic resonance imaging and subsequent development of sacroiliitis on plain radiography. A prospective, longitudinal study. J Rheumatol. 1999; 26(9): 1953-8.

16. Svejgaard A. PP and LPR. HLA and disease suceptibility: clinical implications. Clin Immunol Allergy 4. 1984; 567-80.

17. Breur-Vriesendorp BS, Dekker-Saeys AJ, Ivanyi P. Distribution of HLA-B27 subtypes in patients with ankylosing spondylitis: the disease is associated with a common determinant of the various B27 molecules. Ann Rheum Dis. 1987; 46(5): 353-6.

18. Khan MA, Kellner H. Immunogenetics of spondyloarthropathies. Rheum Dis Clin North Am. 1992; 18(4): 837-64.

19. Khan MA. Ankylosing spondylitis: introductory comments on its diagnosis and treatment. Ann Rheum Dis. 2002; 61 Suppl 3: iii3-7.

20. Sieper J, Braun J, Rudwaleit M, Boonen A, Zink A. Ankylosing spondylitis: an overview. Ann Rheum Dis. England. 2002; 61 Suppl 3: iii8-18.

21. Zochling J, van der Heijde D, Burgos-Vargas R, et al. ASAS/EULAR recommendations for the management of ankylosing spondylitis. Ann Rheum Dis. 2006; 65(4): 442-52.

22. Van Gaalen FA. Does HLA-B*2706 protect against ankylosing spondylitis? A meta-analysis. Int J Rheum Dis. 2012; 15(1): 8-12.

23. Braun J, Bollow M, Remlinger G, et al. Prevalence of spondylarthropathies in HLA-B27 positive and negative blood donors. Arthritis Rheum. 1998; 41(1): 58-67.

24. Brown MA, Kennedy LG, MacGregor AJ, et al. Susceptibility to ankylosing spondylitis in twins: the role of genes, HLA, and the environment. Arthritis Rheum. 1997; 40(10): 1823-8.

25. Laval SH, Timms A, Edwards S, et al. Whole-genome screening in ankylosing spondylitis: evidence of non-MHC genetic-susceptibility loci. Am J Hum Genet. 2001; 68(4): 918-26.

26. Allen RL, Bowness P, McMichael A. The role of HLA-B27 in spondyloarthritis. Immunogenetics. 1999; 50(3-4): 220-7.

27. Liu X, Hu LH, Li YR, Chen FH, Ning Y, Yao QF. The association of HLA-B*27 subtypes with ankylosing spondylitis in Wuhan population of China. Rheumatol Int. 2010; 30(5): 587-90.

28. Liu Y, Jiang L, Cai Q, et al. Predominant association of HLA-B*2704 with ankylosing spondylitis in Chinese Han patients. Tissue Antigens. 2010; 75(1): 61-4.

29. Garcia-Fernandez S, Gonzalez S, Mina Blanco A, et al. New insights regarding HLA-B27 diversity in the Asian population. Tissue Antigens. 2001; 58(4): 259-62.

30. Gonzalez S, Garcia-Fernandez S, Martinez-Borra J, et al. High variability of HLA-B27 alleles in ankylosing spondylitis and related spondyloarthropathies in the population of northern Spain. Hum Immunol. 2002; 63(8): 673-6.

31. Mou Y, Wu Z, Gu J, et al. HLA-B27 polymorphism in patients with juvenile and adult-onset ankylosing spondylitis in Southern China. Tissue Antigens. 2010; 75(1): 56-60.

32. Meryem A, Noureddine B, Ouafaa MS, et al. HLA-B27 subtypes distribution among moroccan patients with ankylosing spondylitis. Am J Med Biol Res. 2013; 1(1): 28-32.

33. Ball EJ, Khan MA. HLA-B27 polymorphism. Joint Bone Spine. 2001; 68(5): 378-82.

34. Lopez-Larrea C, Sujirachato K, Mehra NK, et al. HLA-B27 subtypes in Asian patients with ankylosing spondylitis. Evidence for new associations. Tissue Antigens. 1995; 45(3): 169-76.

35. Choo SY, Antonelli P, Nisperos B, Nepom GT, Hansen JA. Six variants of HLA-B27 identified by isoelectric focusing. Immunogenetics. 1986; 23(1): 24-9.

36. Choo SY, Fan LA, Hansen JA. A novel HLA-B27 allele maps B27 allospecificity to the region around position 70 in the alpha 1 domain. J Immunol. 1991; 147(1): 174-80.

37. Sampaio-Barros PD, Bertolo MB, Kraemer MH, Neto JF, Samara AM. Primary ankylosing spondylitis: patterns of disease in a Brazilian population of 147 patients. J Rheumatol (Canada). 2001; 28(3): 560-5.

38. Reveille JD, Ball EJ, Khan MA. HLA-B27 and genetic predisposing factors in spondyloarthropathies. Curr Opin Rheumatol. 2001; 13(4): 265-72.

39. Koh WH, Boey ML. Ankylosing spondylitis in Singapore: a study of 150 patients and a local update. Ann Acad Med Singapore. 1998; 27(1): 3-6.

40. Londono J, Romero-Sanchez MC, Torres VG, et al. Associação entre os níveis séricos de potenciais biomarcadores com a presença de fatores relacionados à atividade clínica e ao mau prognóstico em espondiloartrites. Rev Bras Reumatol. [Internet]. 2012; 52(4): 529-44. Available at: http://www.scielo.br/pdf/rbr/v52n4/v52n4a06.pdf.

41. Allen RL, Bowness P, McMichael A. The role of HLA-B27 in spondyloarthritis. Immunogenetics. 1999; 50(3-4): 220-7.

42. Conjunto de baixa resolução AllSet+™ Gold B27. Instruções de utilização para identificar a presença ou a ausência de alelos HLA B27. [Internet]. Available at: http://www.onelambda.com/content/dam/onelambda/en/TDX/Documents/securedocs/docs/Product_Insert/MAN0002577_AllSet+ Gold HLA B27 SSP Kit Instructions for Use - Portuguese Translation_rev07.pdf.

43. McGinnis MD, Conrad MP, Bouwens AG, Tilanus MG, Kronick MN. Automated, solid-phase sequencing of DRB region genes using T7 sequencing chemistry and dye-labeled primers. Tissue Antigens. 1995; 46(3): 173-9.

44. Testes de tipagem LABType® SSO. [Internet]. Available at: http://www.onelambda.com/content/dam/onelambda/en/TDX/Documents/securedocs/docs/Product_Insert/RSSO-LTYP-PI-PT-00.pdf.

45. Kulkarni B, Mohanty D, Ghosh K. Frequency distribution of human platelet antigens in the Indian population. Transfus Med. England; 2005 Apr; 15(2): 119-24.

46. Nathalang O, Tantimavanich S, Nillakupt K, Arnutti P, Jaruchaimontree C. HLA-B27 testing in Thai patients using the PCR-SSP technique. Tissue Antigens. 2006; 67(3): 233-6.

47. Parasannanavar DJ, Rajadhyaksha A, Ghosh K. Application of a simple in-house PCR-SSP technique for HLA-B* 27 typing in spondyloarthritis patients. Arthritis. [Internet]. 2013. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24490069. PubMed PMID: 24490069.

48. Sérgio Fraco. Bioinforme. HLA-B27. [Internet]. Available at: http://www.sergiofranco.com.br/bioinforme/index.asp?cs=Hematologia&ps=hlab27.

49. John SW, Weitzner G, Rozen R, Scriver CR. A rapid procedure for extracting genomic DNA from leukocytes. Nucleic Acids Res. 1991; 19(2): 408.

50. Frankenberger BL, Breitkopf S, Albert E, et al. Routine molecular genotyping of HLA-B27 in spondyloarthropathies overcomes the obstacles of serological typing and reveals an increased B *2702 frequency in ankylosing spondylitis. J Rheumatol. 1997; 24(5): 899-903.

CORRESPONDING AUTHOR

Jeane Eliete Laguila Visentainer
PhD Laboratório de Imunogenética; Departamento de Ciências Básicas da Saúde; Universidade Estadual de Maringá (UEM); Av. Colombo, 5.790; CEP: 87020-900; Phone: +55 (44) 3011-5392; e-mail: jelvisentainer@uem.br.